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Myopes have significantly higher serum melatonin concentrations than non-myopes

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Abstract

Purpose: Experimental animal models of myopia demonstrate that higher melatonin (Mel) and lower dopamine (DA) concentrations actively promote axial elongation. This study explored the association between myopia and serum concentrations of DA and Mel in humans.

Methods: Morning serum concentrations of DA and Mel were measured by solid phase extraction-liquid chromatography-tandem mass spectrometry from 54 participants (age 19.1 ± 0.81 years) in September/October 2014 (phase 1) and March/April 2016 (phase 2). Axial length (AL), corneal radii (CR) and spherical equivalent refraction (SER) were also recorded. Participants were defined as myopic if non-cycloplegic spherical equivalent refractive error ≤−0.50 DS at phase 1.

Results: Nine participants were lost to follow up. Mel concentrations were measurable for all myopes (phase 1 $n = 25$, phase 2 $n = 22$) and non-myopes (phase 1 $n = 29$, phase 2 $n = 23$). SER did not change significantly between phases ($p = 0.51$). DA concentrations were measurable for fewer myopes (phase 1 $n = 13$, phase 2 $n = 12$) and non-myopes (phase 1 $n = 23$, phase 2 $n = 16$). Myopes exhibited significantly higher Mel concentrations than non-myopes at phase 1 (Median difference: 10 pg mL^{-1}, $p < 0.001$) and at phase 2 (Median difference: 7.3 pg mL^{-1}, $p < 0.001$) and lower DA concentrations at phase 2 (Median difference: 4.7 pg mL^{-1}, $p = 0.006$). Mel concentrations were positively associated with more negative SER (all $r \geq -0.53$, all $p < 0.001$), longer AL (all $r \geq 0.37$, all $p \leq 0.008$) and higher AL/CR ratio (all $r \geq 0.51$, all $p < 0.001$).

Conclusion: This study reports for the first time in humans that myopes exhibit higher serum Mel concentrations than non-myopes. This may indicate a role for light exposure and circadian rhythm in the human myopic growth mechanism. Further research should focus on younger cohorts exhibiting more dynamic myopic progression and explore the profile of these neurochemicals alongside evaluation of sleep patterns in myopic and non-myopic groups.

Introduction

Myopia presents an economic burden both in terms of the cost of refractive correction and the increased risk of visual impairment arising from associated pathology including glaucoma[1] and chorioretinal atrophy.[2,3] Myopia is a growing health concern[4] with global estimates indicating the number of cases of myopia will reach 324 million by 2025 resulting in an increase in the prevalence of pathological scleral and choroidal degenerations associated with high myopia.[3] Much attention has been directed towards understanding the risk factors associated with myopia and the development of interventions to reduce the incidence and progression of myopia in childhood.[5–8] Research has

demonstrated that many factors contribute to the onset and progression of myopia; genetics, lifestyle and visual environment have all been shown to have significant roles[9–11] and manipulation of the latter two factors hold promise for reducing incidence and progression. To-date the most promising interventions include modifying the image profile projected to the peripheral retina,[12,13] increasing the amount of time a child spends outdoors,[7,14,15] and application of pharmacological agents such as adenosine antagonist 7-methylxanthine (7-mx)[16] or the anti-cholinergic agent atropine[8]. The latter has recently been shown to cause thickening of the choroid in young children (aged 5–10 years).[17] The mechanism by which these visual, environmental and pharmacological interventions influence refractive status are currently unclear.

Circadian rhythms contribute to the control of ocular physiological process and have been demonstrated within both mammalian and non-mammalian ocular tissue including the cornea and retina.[18,19] Circadian rhythms have also been reported in axial length and choroidal thickness in the human eye.[20–22] Stone *et al.*[22] propose that disruption of retinal circadian rhythm may be a key element promoting dysregulation of eye growth and hence myopia. Indeed, Weiss and Schaeffel[23] report that form deprived chick eyes do not demonstrate the same circadian fluctuation in axial length as control eyes; axial length increased at a faster rate during the night than during the day in form deprived eyes. Nickla *et al*[24] demonstrated that disruption of circadian rhythm in chicks, through exposure to two hours of bright light during the night, leads to abolition of diurnal variations in choroidal thickness and axial elongation. Furthermore, Bertolet *et al.*[25] reported that in healthy young adults, choroidal thickness was significantly greater at 6 pm than at noon in emmetropes but this diurnal variation was not reported in myopes. This relation between circadian rhythm and myopia is not limited to the eye; a recent report by Ayaki *et al.*[26] describes poorer sleep quality in highly myopic children and young adults when compared with less myopic or emmetropic peers.

Systemic circadian rhythms are primarily regulated by the hypothalamic suprachiasmatic nucleus in the brain which also control the circadian release of the neurohormone melatonin (Mel) and the neurotransmitter dopamine (DA). Mel concentrations are greatest during the night and DA concentrations are greatest during the day. The synthesis of Mel primarily occurs in the pineal gland in humans[27] and has also been documented in ocular cells and structures including retinal photoreceptors in the frog eye[28] and ciliary epithelial cells in human eyes.[29] Three Mel receptors (Mel_{1a}, Mel_{1b} and Mel_{1c}) have been located throughout the retina, the sclera and the cornea of the frog,[30,31] the choroid, the retina and the sclera of the chick eye[32,33] and retinal ganglion cells and inner nuclear layers of guinea pig eyes.[33]

Melanopsin is a blue light-sensitive photopigment whose synthesis is partially modulated by Mel.[34] Melanopsin can be found within intrinsic photosensitive retinal ganglion cells within the mammalian eye.[35] These intrinsically photosensitive retinal ganglion cells innervate the hypothalamic suprachiasmatic nucleus within the brain, contributing to the entrainment of the light mediated circadian clock (circadian photoentrainment).[36] They also contribute to light mediated responses within the eye, including the pupillary light response, and influence the activity of dopaminergic retinal cells.[37,38] Schaeffel *et al.*[39] explored the association between refractive error and melanopsin signal strength in adults aged 18–87 years. The differential in recovery time of the pupillary response to blue and red light was used to indicate melanopsin response but no association was found with refractive error. No research has yet explored the association between Mel and refractive error in humans.

Although DA is primarily regarded as a cerebral neurotransmitter, it is also released from retinal type 2 amacrine and interplexiform cells within the mammalian eye.[40] Its functions within the eye are numerous including involvement in retinal light adaptive processes[40,41] and retinal pigment epithelium physiology.[40]The functions of DA are mediated by D_1 and D_2 receptor families. The D_1 receptor family (D_1 and D_5 receptors) have been shown to be predominantly located in the retina within bipolar, horizontal, amacrine and ganglion retinal cells in mammalian and chick eyes.[40,42] The D_2 receptor family (D_2, D_3 and D_4 receptors) have been shown to be predominantly located within the RPE in the chick eye[43,44] and, specifically, within the photoreceptor layer in the human eye.[45]

Mel and DA form a mutual inhibitory relationship whereby Mel negatively influences DA release in both neural and ocular tissue, including the retina. Previous reports have explored the role of Mel and DA in animal models of myopia. Lower retinal DA concentrations are reported in experimental chick myopia.[46–48] Stone *et al*[48] demonstrate that DA retinal synthesis is reduced in one-day old form deprived chick eyes and the authors propose that DA may contribute to the regulation of emmetropization and normal ocular growth. Furthermore, the enrichment of DA concentrations using DA agonist (apomorphine) eye drops retards the development of form deprivation myopia in primates[49] and in chicks.[48] Similarly, in guinea pig eyes, Dong *et al.*[50] demonstrated that application of subconjunctival injections of apomorphine inhibits the development of form deprivation myopia.

Although ocular growth is believed to be locally regulated,[51] circulating blood concentrations of DA and Mel are likely to influence the highly vascular ocular tissues and hence ocular growth. This has previously been evidenced in

chicks in which systemic administration of Mel promoted choroidal thinning[32] and in guinea pigs where the systemic injection of a precursor of DA (levodopa) retarded the development of form deprivation myopia.[52] Ocular Mel and systemic circulating Mel concentrations have also been shown to be associated in the frog[53] and Newt eye.[54]

While animal studies indicate that refractive status can be manipulated with systemic and ocular administration of Mel and DA there are no previous studies investigating the association between Mel and DA with myopia in humans. The aim of this prospective, observational study was to explore the association between myopia and serum concentrations of DA and Mel in a human population for the first time.

Materials and methods

Initial measures were completed in September/October 2014 (phase 1) and repeated 18 months later in March/April 2016 (phase 2). This facilitated the exploration of seasonal variation in Mel and DA concentrations. Participants were aged 18–20 years at phase 1 and were recruited from first year undergraduate students attending Ulster University (Coleraine campus (55°N).

Participants with a diagnosed medical condition or taking prescribed medication known to affect DA or Mel concentrations, such as levodopa and Mel, were excluded. Ethical approval was granted from the Ulster University Research Ethics Committee (REC/14/0003) and written informed consent was obtained from all participants before commencing the study protocol and after explanation of the nature and possible consequences of the study. Research adhered to the tenets of the Declaration of Helsinki. All blood samples were processed and stored in accordance with the Human Tissue Act 2004.

Autorefraction, ocular biometry and parental myopia

Non-cycloplegic autorefraction was completed using the Shin-Nippon SRW-5000 binocular open field autorefractor (Shin-Nippon, Tokyo, Japan) while the participant viewed a distance target. The representative value from each eye was determined by the instrument and the average of both eyes used in analysis. Participants were defined as myopic if the spherical equivalent refraction (SER) equated to less than or the equivalent of −0.50 dioptre sphere (DS).[55]

Axial length and corneal radii (CR) were measured using the IOL Master (https://www.zeiss.com/meditec/int/products/ophthalmology-optometry/cataract/diagnostics/optical-biometry/iolmaster-500.html). A total of five AL measures with a signal-to-noise ratio of greater than two were measured from each eye. The average of both eyes was used in analysis. A total of three CR measures were also recorded from each eye and the average of both eyes included in analysis. The AL to CR ratio (AL/CR) was determined from these measures and an average value was derived from both eyes for each participant.

Data on parental myopia was determined from a validated refractive status questionnaire[56] and categorised as either '0 parents myopic', '1 parent myopic' or 'both parents myopic'.

Blood collection

Circulating serum concentrations of Mel and DA were determined from fasting blood samples. Participants were required to fast from 10 pm the previous evening. A 4 mL serum blood sample (https://shop.gbo.com/en/row/products/preanalytics/venous-blood-collection/vacuette-tube/serum/) was collected from the antecubital vein between 8.30 am and 10 am. Sampling times were restricted in this way to reduce inter and intra-participant variation in Mel and DA arising from circadian rhythm and daylight exposure.[57]

Serum samples were centrifuged at 2200 g for 15 min at 4°C (http://www.mseuk.co.uk/Products/Centrifuges/Refrigerated/Harrier_18_80R/Default.aspx) within two hours of collection and 1000 μL of serum was isolated from the centrifuged sample. The analysis of DA from phase 1 serum samples indicated that this analyte was not as readily detected as Mel. Therefore, serum samples from phase 2 intended for DA analysis were preserved in a final concentration of 0.1% ascorbic acid solution 0.1 M hydrochloric acid to prevent degradation of this analyte. All samples were stored at −80°C prior to analysis.

DA and Mel analysis

DA and Mel were quantified using liquid chromatography followed by on-line solid phase extraction and tandem mass spectrometry analysis (LC-On-Line SPE-MS/MS).

Quantification was performed on an API 4000 (AB Sciex, Warrington, UK) coupled to a Shimadzu LC system consisting of a controller (CBM-2A), auto sampler (SIL-20ACxr), LC pumps (20AD xr,) loading pump (20ADsp) and column oven (CTO-20A) (Shimadzu, Kyoto, Japan). It was operated using Analyst software (version 1.6.1, AB Sciex, Warrington, UK). The limit of quantitation (LOQ) for Mel was 2 pg mL^{-1} (signal/noise (S/N) = 32.1, Coefficient of Variation (CV) (n = 3) = 3.45%) and the LOQ for DA was 10×10^3 pg mL^{-1} (S/N = 23.3, CV, (n = 3) = 4.6%).

The values for intra- and inter- assay precision and accuracy were acceptable (<20% Relative Standard Deviation (RSD) for precision and ±20% accuracy) at the LOQ of 2 pg mL^{-1} for Mel and LOQ of 10×10^3 pg mL^{-1} for

DA. Acceptable reproducibility for DA and Mel measures upon repeat injections was demonstrated with a RSD of 2.2% for DA and 4% for Mel in serum samples.[58]

Statistical methods: sample size

Owing to the novel nature of the study, sample size calculations were applied retrospectively to determine statistical power.

Statistical methods: analysis

All statistical tests were performed using Stata 13.1 (StataCorp Texas, USA) using a statistical significance level of 5% throughout ($p < 0.05$).

All measures of Mel were normally distributed as indicated by the Skewness and Kurtosis test for normality (all $p \geq 0.066$). Phase 2 measures of DA were also normally distributed ($p = 0.18$). As phase 1 measures of DA were not normally distributed ($p = 0.041$), data were squared to follow a normal distribution as indicated by the Stata Ladder of powers test.[59] For comparison purposes, graphical data presented pertain to raw data.

DA and Mel concentrations may be subject to seasonal variation[60,61] therefore, data from phase 1 (end of summer) and phase 2 (end of winter) were analysed separately. Spearman's correlation was used to assess the correlation between DA, Mel and continuous ocular biometric variables including; SER, AL, CR and AL/CR. Fisher's exact test was used to assess the relationship between parental myopia and the presence of myopia.

Multiple imputation by chained equations was used to account for missing data arising from participant drop outs.[62] A repeated logistic regression model including the imputed data was used to assess the relationship between the presence of myopia (yes/no) and DA and Mel concentrations. A linear regression model including the imputed data was used to assess the relationship between DA, Mel and change in SER, AL, CR and AL/CR over the 18-month study period.

DA and Mel were included as predictors. A total of 50 imputed datasets were generated for DA and 15 for Mel. Active imputation was used to address missing AL/CR values.

Results

Participant characteristics

A total of 83% of participants with measures at phase 1 ($n = 54$) also had available data at phase 2 ($n = 45$). Of the nine participants who dropped out; five participants had left the University, three participants were non-contactable and one participant did not want to have a repeat blood sample taken. The baseline characteristics of those who dropped out did not significantly differ by SER (d.f. = 52, $p = 0.47$), parental myopia ($\chi^2 = 3.1$, $p = 0.079$) or by gender ($\chi^2 = 0.58$, $p = 0.45$) from those who did not drop out. Myopes (SER ≤ -0.50 DS) and non-myopes (SER > 0.50 DS) were classified from refractive data collected at phase 1. *Table 1* summarises the available refractive and ocular biometric data at each phase for myopes and non-myopes.

Spherical equivalent refraction was relatively stable over the study period in both the myopic (mean change SER: -0.22 ± 0.27DS) and the non-myopic (mean change SER: 0.01 ± 0.43DS) groups and change in SER was not statistically significant in either group (all $p \geq 0.079$). Participants who were classified as non-myopic or myopic at phase 1 remained within their respective refractive status categories throughout the study period.

DA and Mel: characteristics

Serum samples were collected from each participant at approximately the same time (mean difference between phase 1 and phase 2 = 30.3 min (standard deviation (S.D.): 8.2) min). Mel was detectable within all serum samples analysed at phase 1 and phase 2. Of the 54 samples analysed at phase 1, DA concentrations were detectable for 36 participants (66%). Of the 45 samples at phase 2 containing the ascorbic acid stabiliser, DA concentrations were detectable for 28 participants (62%). There was no statistically significant seasonal variation in Mel ($p = 0.75$) or DA ($p = 0.31$). DA was inversely correlated with Mel at phase 1

Table 1. Summary statistics describing refractive and ocular biometric values of myopes and non-myopes at each phase

Average right and left eye (Mean (±S.D.))	Myopes ($n = 25$)	Non-myopes $n = 29$	d.f.[a]	p
Phase 1				
SER (DS)	−2.37 ± 1.27	+0.62 ± 0.89	52	<0.001
AL (mm)	24.7 ± 0.90	23.3 ± 0.80	52	<0.001
CR (mm)	7.8 ± 0.26	7.9 ± 0.28	52	0.29
AL/CR	3.2 ± 0.09	2.9 ± 0.09	52	<0.001
	Myopes ($n = 22$)	**Non-myopes ($n = 23$)**		
Phase 2				
SER (DS)	−2.34 ± 1.12	+0.78 ± 1.16	41	<0.001
AL (mm)	24.7 ± 0.76	23.4 ± 0.81	41	<0.001
CR (mm)	7.8 ± 0.24	8.0 ± 0.26	41	0.036
AL/CR	3.2 ± 0.09	2.9 ± 0.08	41	<0.001

SER, Spherical equivalent refraction; AL, Axial length; CR, Corneal curvature; AL/CR, Axial length to corneal radii ratio.
[a]Independent samples *t*-test.

($\rho = -0.72$, $p < 0.001$) and at phase 2 ($\rho = -0.49$, $p = 0.009$).

DA, Mel and myopia (SER)

Parental myopia was significantly associated with a more negative SER ($F(2,45) = 3.56$, $p = 0.037$) but it was not associated with the presence of myopia ($p = 0.25$). Therefore, parental myopia was only accounted for in subsequent analysis of continuous refractive data.

When considered as a group and compared with non-myopes, myopes exhibited significantly higher Mel concentrations than non-myopes at phase 1 and at phase 2 (*Table 2* and *Figure 1*). This association was also significant after accounting for missing data in the repeated logistic regression model (OR = 1.1, 95% CI = 1.0–1.1, $p = 0.031$). Although myopes also exhibited significantly lower DA concentrations than non-myopes at phase 2 this association was not significant at phase 1 (*Table 2* and *Figure 1*) nor when missing data in the repeated logistic regression model were accounted for (OR = 1.0, 95% CI = 1.0–1.0, $p = 0.39$).

Spherical equivalent refraction was negatively associated with Mel at phase 1 ($\rho = -0.53$, $p < 0.001$) and at phase 2 ($\rho = -0.61$, $p < 0.001$) and this remained significant after controlling for parental myopia (all $p \leq 0.001$) (*Figure 2*). DA was not associated with SER at phase 1 ($\rho = -0.16$, $p = 0.36$). Although DA was positively associated with SER at phase 2 ($\rho = 0.44$, $p = 0.028$) following univariate analyses (*Figure 2*), this association did not remain significant after accounting for parental myopia in multivariate analyses ($p = 0.16$).

DA, Mel and ocular biometry

The association between DA, Mel and ocular biometry is summarised in *Table 3*. Mel was positively associated with a longer AL and higher AL/CR at phase 1 and phase 2 (*Figure 3*) but there was no association between Mel and CR at either phase. DA was not significantly associated with any of these ocular measures.

DA, Mel and changes in refraction and ocular biometry

There was no significant seasonal variation in Mel concentrations. Mel concentrations were not associated with change in SER, AL or AL/CR between phase 1 and phase 2 amongst myopes (all $p \geq 0.47$) or non-myopes (all $p \geq 0.11$).

Serum DA concentrations were also not significantly associated with change in SER, AL or AL/CR between phase 1 and phase 2 amongst myopes (all $p \geq 0.092$) or non-myopes (all $p \geq 0.10$).

Discussion

The present study describes a positive association between the magnitude of myopia and morning serum concentrations of Mel. Myopes demonstrated a median Mel concentration that was up to three-times greater than the median Mel concentration observed in the non-myopic group. The association found between Mel and myopic refractive error is further supported by positive associations between Mel, AL and AL/CR; most strongly between Mel and AL/CR. Morning serum concentrations of DA were not significantly different between myopes and non-myopes. While our results show an association between refractive status and serum Mel, our data cannot be used to indicate a causal relationship. Our findings align with animal models of experimentally induced myopia in which systemic and ocular application of Mel has been shown to impact on refractive status and ocular shape[32] and suggest that Mel may influence human refractive error and ocular shape.[32,49,50,52,63] Further research is required to determine the nature of this influence.

Table 2. Summary statistics indicating that myopes exhibited higher melatonin (Mel) concentrations than non-myopes at each phase and lower dopamine (DA) concentrations than non-myopes at phase 2

	Mel (pg mL^{-1}) (Median(Interquartile range))				DA(pg mL^{-1}) (Median(Interquartile range))			
Phase	**Myopes**	**Non-myopes**	**d.f.[a]**	***p***	**Myopes**	**Non-myopes**	**d.f.[a]**	***p***
1	15.5 (11.9–19.9) $n = 25$	5.5 (3.7–7.4) $n = 29$	52	<0.001	12.1×10^3 (11.0×10^3–14.4×10^3) $n = 13$	16×10^3 (13.0×10^3–20.6×10^3) $n = 23$	34	0.085
2	14.2 (10.1–16.6) $n = 22$	6.9 (4.1–9.1) $n = 23$	48	<0.001	13.1×10^3 (11.8×10^3–15.3×10^3) $n = 12$	17.8×10^3 (13.4×10^3–21.3×10^3) $n = 16$	26	0.006

[a]Independent samples *t*-test.

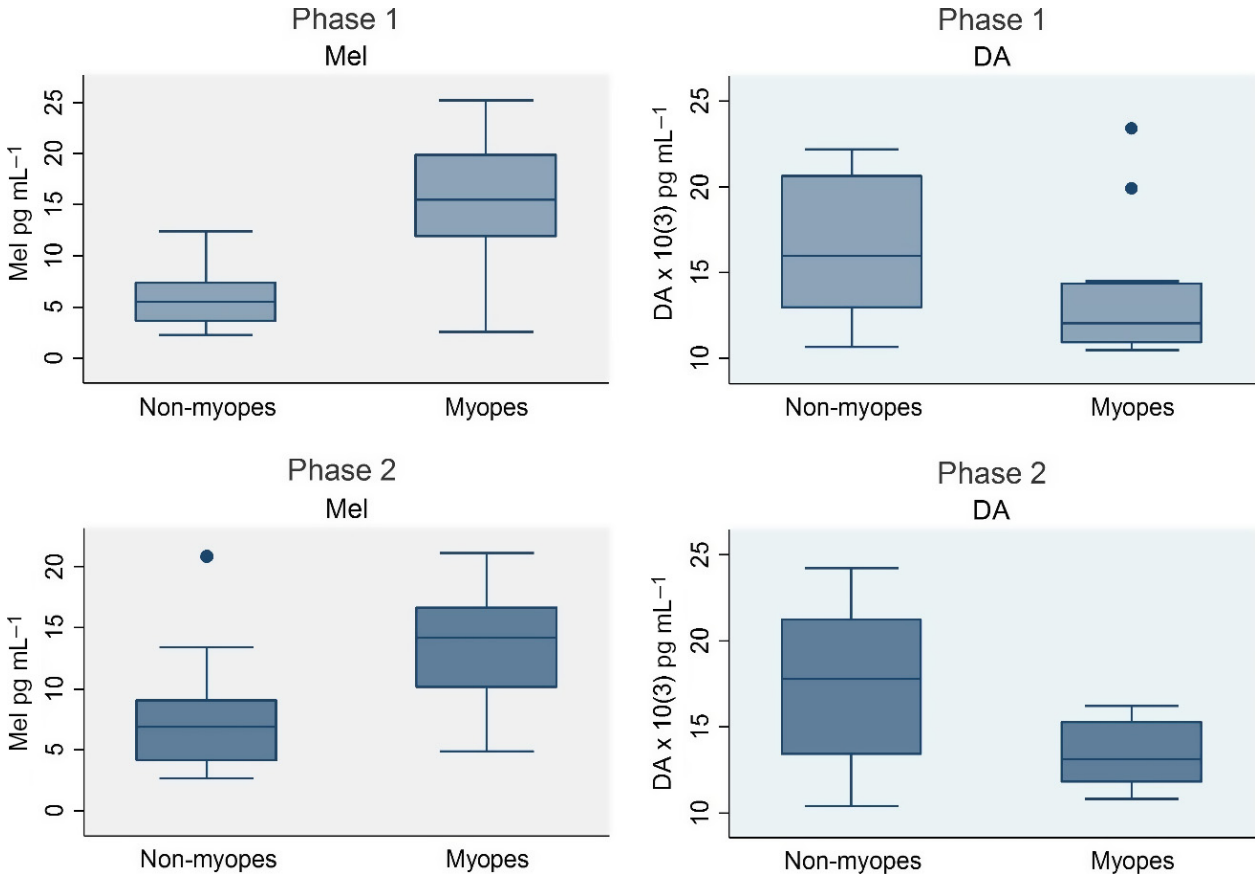

Figure 1. Box plots illustrating significantly higher Mel concentrations in myopes than non-myopes at both phases (phase 1: d.f. = 52, <0.001, P2: d.f. = 43, $p < 0.001$) and significantly lower DA concentrations than non-myopes at phase 2 only (phase 1: d.f. = 34, $p = 0.085$, P2: d.f. = 26, $p = 0.006$). Individual points indicate outliers. Phase 1: (Median (Interquartile range)): Mel: Myopes:15.5 pg mL^{-1} (11.9–19.9 pg mL^{-1}) Non-myopes:5.5 pg mL^{-1} (3.7–7.4 pg mL^{-1}). DA: Myopes: 12.1 × 10^3 (11.0 × 10^3–14.4 × 10^3) Non-myopes: 16 × 10^3 (13.0 × 10^3–20.6 × 10^3). Phase 2: (Median (Interquartile range)): Mel: Myopes:14.2 pg mL^{-1} (10.1–16.6 pg mL^{-1}) Non-myopes:6.9 pg mL^{-1} (4.1–9.1 pg mL^{-1}). DA: Myopes: 13.1 × 10^3 pg mL^{-1} (11.8 × 10^3–15.3 × 10^3 pg mL^{-1}) Non-myopes: 17.8 × 10^3 pg mL^{-1} (13.4 × 10^3–21.3 × 10^3 pg mL^{-1}). [Colour figure can be viewed at wileyonlinelibrary.com]

It is known that circulating concentrations of Mel are higher in younger individuals[64] and that myopia generally emerges in the pre-teenage years and exhibits more active progression with earlier onset.[65] It would be valuable to investigate serum concentrations of DA and Mel in younger participants undergoing more dynamic refractive change to ascertain whether these neurochemicals are linked to refractive status in incipient myopes and possibly predictive for refractive change and ocular biometric growth.

The finding that morning serum concentrations of Mel in adult myopes are significantly higher than in non-myopic individuals may also be important in the context of possible treatments or preventative regimes for human myopia, if these findings are indeed replicated in younger individuals. Spending more time outdoors[7,14] has been shown to protect against myopia and myopic progression in childhood and this activity may have its therapeutic effect, at least in part, through strengthening of circadian rhythms. There is emerging evidence that the human myopic eye has atypical circadian rhythms[25] and this feature of myopia warrants further investigation in the context of understanding the role of circadian rhythms and refractive development.

Strengths and limitations

Mel is more efficiently ionised and therefore more sensitively detected using the LC-On-Line SPE-MS/MS method than DA. The lower success rates for measuring DA in serum samples resulted in the study being under-powered for exploring differences between the myopic and non-myopic groups as indicated by retrospective sample size calculations (power of 90%, significance 5%) using the outcomes from the current study and when using data from

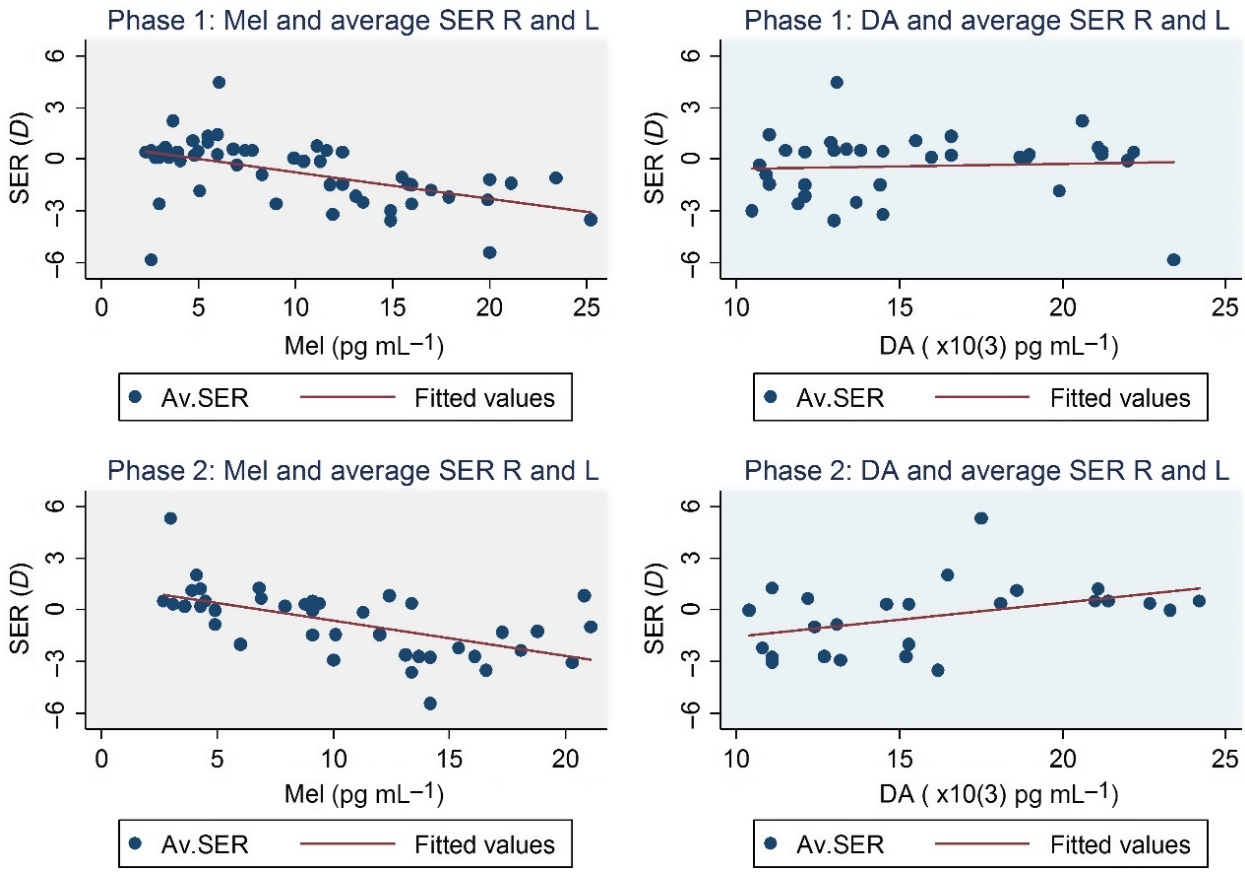

Figure 2. Scatter graphs illustrating the association between Mel, DA and SER. Phase 1*: Mel:* ρ = −0.53, $p < 0.001$, $R^2 = 0.27$. *DA:* ρ = 0.16, $p = 0.36$, $R^2 = 0.004$. Spearman's corr. Phase 2: *Mel:* ρ = −0.61, $p < 0.001$, $R^2 = 0.34$. *DA:* ρ = 0.44, $p = 0.028$, $R^2 = 0.17$. Spearman's corr. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3. Table summarising the association between dopamine (DA), melatonin (Mel) and ocular biometric measures at each phase. Mel was positively associated with axial length (AL) and axial length to corneal radii ratio (AL/CR) (all $p \leq 0.008$) but not with corneal curvature (all $p \geq 0.059$)

	Mel				**DA**			
	Phase 1		**Phase 2**		**Phase 1**		**Phase 2**	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
AL[a]	0.37	0.008	0.52	<0.001	−0.21	0.22	−0.24	0.26
CR[a]	−0.05	0.73	−0.28	0.059	−0.19	0.28	0.07	0.74
AL/CR[a]	0.51	<0.001	0.71	<0.001	−0.10	0.56	−0.38	0.054

[a]Spearman's Correlation.

previous inter-group comparisons of DA serum concentrations.[66] However, circulating serum concentrations of DA and Mel are known to be inversely and strongly related[67] and this was borne out in the present results where data for both neurochemicals were available from single individuals; where Mel concentrations were relatively high, DA concentrations were relatively low. Further work quantifying DA concentrations from blood samples taken during the middle of the day, when DA concentrations are known to be at their peak,[40,68] rather than early in the morning, may be more successful in quantifying DA concentrations and hence yield larger data sets with which to compare refractive groups.

Fasting blood samples were taken between 8.30 and 10.00 am to reduce inter- and intra-participant variation in the diurnally phasic neurochemicals under investigation. However, no information on sleep patterns, time of waking, extent of exposure to daylight prior to blood sampling

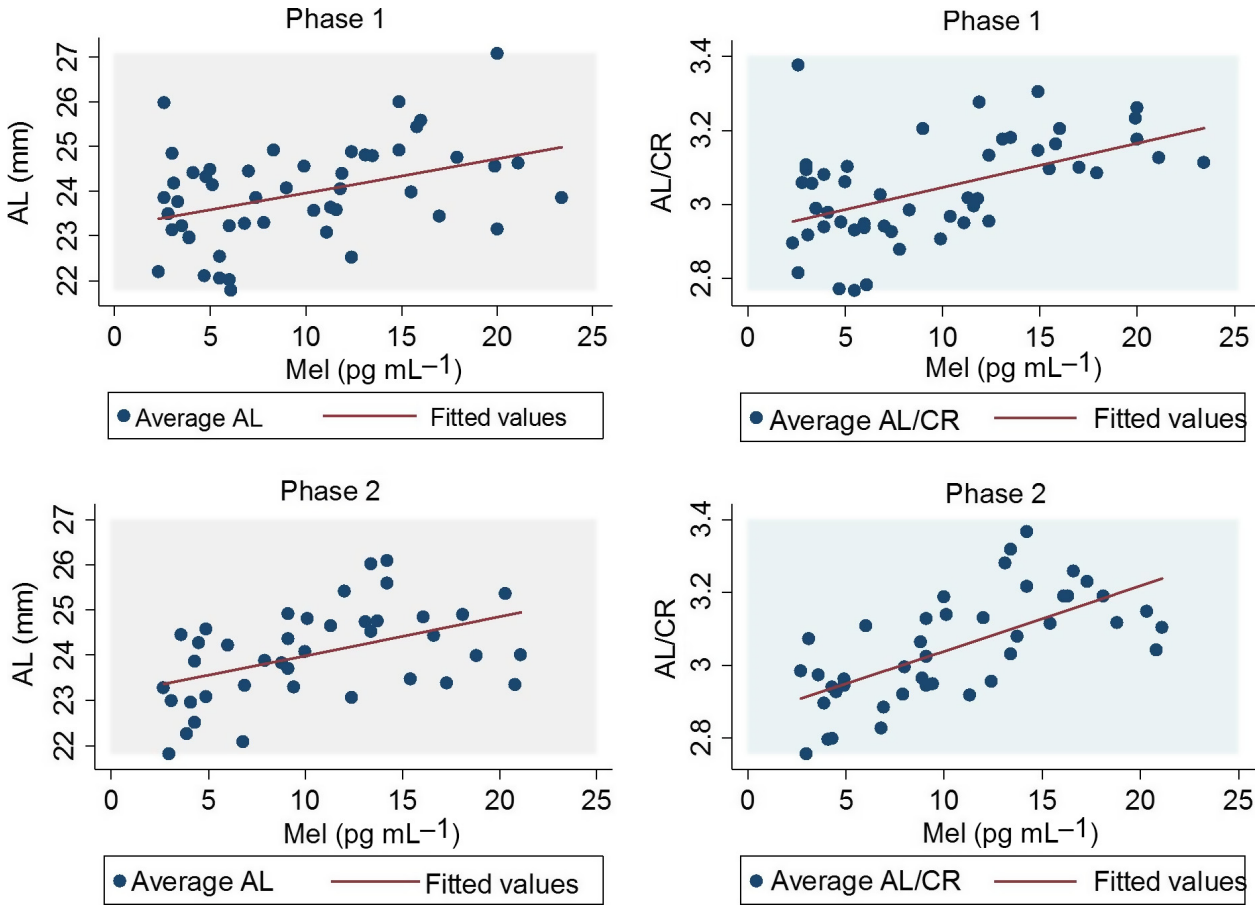

Figure 3. Scatter graphs illustrating a positive association between Mel, AL and AL/CR. Phase 1: AL: $\rho = 0.37$, $p = 0.008$, $R^2 = 0.16$. AL/CR: $\rho = 0.51$, $p < 0.001$, $R^2 = 25$; Spearman's corr. Phase 2: AL: $\rho = 0.52$, $p < 0.001$, $R^2 = 0.22$. AL/CR: $\rho = 0.71$, $p < 0.001$, $R^2 = 44$; Spearman's corr. [Colour figure can be viewed at wileyonlinelibrary.com]

or menstrual cycle was obtained from participants. These are factors which have previously been shown to influence Mel status. However, the lack of control of these factors applies equally to the myopic and non-myopic participants and is unlikely to undermine the almost three-fold average difference between the myopic and non-myopic participants. Additionally, while previous research has identified that night-time measures of Mel are influenced by season in Finland[69] where the variation in day length varies from five hours in the winter to 22 h in the summer, our data were collected in the morning and at a latitude which experiences less extreme variation in day length.[70] This may explain the lack of seasonal variation in Mel found in the present study, consistent with data collected at similar latitude in Germany.[71] Seasonal variation in Mel may be more strongly associated with more northerly latitudes and night-time measurement protocols. The menstrual cycle has been reported to influence night-time measures of Mel and this factor should be considered in further work involving night-time samples.[69]

Differences in sleep patterns between myopes and non-myopes may have contributed to the difference in Mel serum concentrations reported in the present study. Sleep quality was recently shown to be reduced in highly myopic children[26] with parents reporting that such children had a shorter sleep duration and a later bedtime. Furthermore, in young adults 12–19 years of age,[72] the risk of myopia showed a moderate decrease with every one hour increase in sleep. It would be valuable in future studies to evaluate sleep quality in association with Mel and DA serum levels.

Traditionally, myopes and non-myopes have often been purported to have differing personality types.[73] These personality traits could influence Mel levels in the two groups under test and no evaluation of personality type was undertaken in the present study. While more recent reports fail to provide robust evidence for personality differences in myopic vs non-myopic individuals,[74] it may be valuable in future studies to consider personality traits in conjunction with biometric data and sleep characteristics.

Refractive error was determined using non-cycloplegic autorefraction. In order to minimise the effects of proximal accommodation and to promote a relaxed accommodative state, a binocular open field autorefractor with a fixation target positioned at 6 m was employed. The measurement of refractive error using the Shin-Nippon open field autorefractor has been reported to be within 0.50 DS of the subjective refraction measured in a cohort of a similar age.[75]

The significant association between morning Mel and refractive and ocular biometric measures should be considered in the context of the *R*-squared values (25–44%) which indicate that Mel may be associated with a moderate amount of the variation in these measures. It is widely held that there are multiple factors influencing refractive development so these moderate *R*-squared values are not surprising.

Conclusion

The results of the present study demonstrate that higher serum concentrations of Mel, measured in the morning, are associated with myopia in young adults. In the context of experimental models of animal myopia and refractive manipulation, and of human intervention studies evaluating treatments to retard the onset and progress of myopia, these results provide a valuable platform for future research into the role of neurochemicals and biological rhythms in the development and control of refractive error.

Disclosure

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

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